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## (54) PROCESS FOR OBTAINING PLANT PEPTONES WITH A HIGH HYDROLYSIS DEGREE AND APPLICATIONS THEREOF

(57) The process includes a first stage to eliminate polyphenols from the starting vegetable isolates, followed by a double hydrolysis treatment, firstly with non-specific endoproteases and then, with specific endoproteases and exoproteases.

The hydrolyzed peptones obtained in this way are applicable to the agroalimentary and medico-pharmaceutical industries, especially in the sectors of human, animal and clinical nutrition.

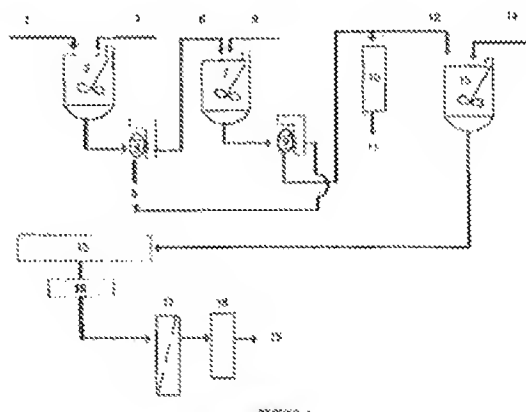


FIGURE 1

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## Description

## TECHNICAL FIELD OF THE INVENTION

[0001] This invention pertains to the technical field of the Agroalimentary and Medico-Pharmaceutical Industries and especially refers to human, animal and clinical nutrition.

[0002] More specifically, the invention provides a process to obtain vegetable peptones with a high degree of hydrolysis of great interest in the mentioned technical sectors.

## STATE OF THE ART PRIOR TO THE INVENTION

[0003] The availability of different types of hydrolyzates in sufficiently large amounts and at reasonable prices would be of great importance and use for the preparation of special diets which, at present, are very expensive and hence, hardly used, although advisable from a medical standpoint.

[0004] Current societies and, particularly, those of developed countries, do not require random hydrolyzates [Parrado, J., Millán, F., Hernández-Pinzón, I., Bautista J and Machado A., 1993, *Process Biochem.*, 28, 109-113; Ziajku S., Dzwolak W and Zubel J., 1994, *Milk Science International*, 49, 7] aimed at general nutrition, culture of micro-organisms, additives for beverages, etc. Nowadays, hydrolyzates are required having well defined compositions and characteristics, either because they are not available on the market or because those existing are very expensive and need to be cheapened. Having hydrolyzates with a defined amino-acid composition [Parrado J., Millán F., Hernández-Pinzón, I., Bautista J and Machado A., 1993, *J. Agr. Food Chem.*, 41, 1821-1825, Bautista J., Hernández-Pinzón I., Alaiiz M., Parrado J and Millán F., 1996, *J. Agr. Food Chem.*, 44, 967-971] is very desirable, both for the nutrition industry and the medico-pharmaceutical ones [Furst P., 1992, *Clin. Nutr.*, 10, 519-529]. Hence, for example, branched aminoacid (AAR) enriched hydrolyzates, either with mixtures of free aminoacids or with peptides rich in AAR, have been successfully used in the treatment of various hepatic pathologies, especially encephalopathies, Grunggreiff K., Kleine F.D., Musie H.E., Dietz U., Franke D., Klanck S., Page I., Kleine S., Losene B and Pfeiffer K.P., 1993, *Z-Claskoenterol*, 31, 235-241; Rossi-Fanelli F., 1990, *Adv. Exp. Med. Biol.*, 272, 227-233].

[0005] The production of proteic hydrolyzates from residues rich in proteins (meat flour) and non-conventional protein sources (vegetable proteins) [Anuario de Estadística Agraria 1996, Edited by the Technical Department of the Ministry of Agriculture, Fisheries and Food; Boatright W.L., Hettiarachchy N.S., 1995 *JAOCS*, 72, 1445-1451; Parades-López O., Ordóñez-Falomir O., Olivares-Vázquez M.R., 1991 *J. Food Sci.*, 53, 1396-1398] is a well established process in the food industry.

[0006] Enzymatic hydrolysis is a very efficient process which, moreover, may be performed under reasonable conditions of temperature, pH and pressure, such that the nutritional quality of the amino-acids is maintained practically unchanged, since the destruction or modification of certain amino-acid residues does not take place, different to the case of chemical hydrolysis. In enzymatic hydrolysis, hydrolyzate neutralization is avoided and hence, the high content in salts which would limit the use of hydrolyzates in certain applications, for example, in diet foods and parenteral preparations.

[0007] In recent years, many enzymatic protein hydrolysis processes have been studied for their application at a commercial scale. The interest for these types of process is large and, although the production of simple proteic hydrolyzates is not a problem, the production of defined hydrolyzates, namely, well defined ones, as demanded by the market, continues to be a problem and represents the greatest challenge in this field. The latter type of hydrolyzate, if possible, should combine the following characteristics: (i) perfectly defined composition in relation to the amino-acids contained (aminogram), as well as their size distribution (% in peptides, oligo-peptides, tetra-tri-di-peptides and free aminoacids); (ii) absence of or a negligible bitter flavour and, (iii) be hypoallergenic.

[0008] The patents existing to obtain proteic hydrolyzates, including both the chemical approach (use of acids and bases) and the enzymatic one (use of enzymes), are general and do not indicate the substrates used nor, in the case of enzymatic hydrolysis, the enzymes employed and of course, do not include the details (Patent 489358, No. 2278/79, Industrial Property Register, Nestlé).

## DETAILED DESCRIPTION OF THE INVENTION

[0009] This invention, as indicated in the title, refers to a process to obtain vegetable peptones with a high degree of hydrolysis.

[0010] More specifically, in this report, the process to obtain enzymatic hydrolyzates of vegetable proteins with a high degree of hydrolysis (GH 50%) is described, specifying, the substrate(s) used [sunflower, rape seeds, chickpeas, lentils, lupins and grape refuse] and the enzymes employed, including their commercial name. The process is the basis to obtain what has been called "customised hydrolyzates".

[0011] The main purpose is to include the preparation of the substrates, which in all cases implies the elimination of the polyphenols. The elimination of the latter, present in all vegetable protein isolates, is necessary because these substances generally inhibit the enzymatic activity of the proteases, such that their elimination is absolutely necessary for the efficient operation of the process.

[0012] The second important purpose is the optimization of the hydrolysis process whose treatment with more than one protease, is based on the resistance to hydrolysis of the allergic "epitopes" present in proteins. For this reason, in an initial treatment, the proteins are hydrolyzed with a) some endoprotease(s) with non-specific activity(ies), such that in a second step, once a practically constant degree of hydrolysis has been reached (12-15% depending on the substrate), a mixture of more specific endoproteases and exoproteases are added together (resulting in degrees of hydrolysis >50%), such that the probabilities of destroying the allergenic "epitopes" is almost total, besides eliminating the possible bitter flavour of some hydrolyzates. The proteolytic enzymes that have been used in each substrate are: alkalase, kerase and flavourzyme for sunflower proteins; L-600 and flavourzyme for rape seed; goglet and flavourzyme for chickpeas, lentils and lupinus proteins; alkalase and kerase for grape refuse proteins. The hydrolyzates are concentrated and micronized to give a dry product.

[0013] Below, the characteristics and specifications of the aforementioned proteolytic enzymes used in this invention are indicated:

- Alkalase: more specifically, alkalase 2.4L, is a bacterial protease, being highly effective and especially developed for the hydrolysis of all types of proteins. It is produced as from a selected strain of *Bacillus Licheniformes*. It is an endopeptidase with an approximate molecular weight of 27,200 daltons. It is easily soluble in water at all concentrations and its density is 1.18 g/ml. It has an activity of 2.4 Anson Units per gram (Au/g), optimum pH of 8 and optimum temperature of 50°C-60°C. It is available in the liquid form and is available from the company, Novo Nordisk Bioindustrial, S.A.
- Flavourzyme: more specifically, flavourzyme 1,000 MG, is produced by the fermentation of *Aspergillus oryzae* and contains endoprotease and exopeptidase activity. The molecular weight differs from the different endoproteases, since it is an endo- and exo-protease complex. It is easily soluble in water, has an activity of 1,000 LAPU/g, optimum pH of 7 and optimum temperature of 50°C. It comes in its granulated form and is available from Novo Nordisk Bioindustrial S.A.
- Kerase: this is a microbial enzyme and is obtained from *Streptomyces Fradiae*. It is a mixture of endo- and exopeptidase, which behave like serin protease and is stable in the presence of oxygen. It is non-toxic, free from antibiotics and bacterial contamination. It is easily soluble in water, has an activity of  $10^{-6}$  Katal units/mg following the Anson method. It has an approximate molecular weight of 20,000 Daltons. The maximum activity pH is 8 and optimum temperature 55°C. It comes in the powder form and is produced by Compañía Española de la Penicilina y Antibióticos, S.a. (CEPA, S.A.).
- L-660: This is a microbial protease serin. It is an endo-protease with an approximate molecular weight of 22,500 Daltons, optimum pH of 10 and optimum temperature of 60°C. It is easily soluble in water, has an activity of 660,000 DU/ml (Dell/ml). It comes in the liquid form and is available from the company MKC.

[0014] According to the above, the process of this invention allows the attainment, at a semi-preparation scale of proteic concentrates and isolates as from agroindustrial waste (sunflower, rape seed, grape refuse, lupin etc.). These proteic concentrates and isolates are the starting point to obtain different types of hydrolyzate, by the use of proteases. Moreover, it is possible to obtain the following at a practical level: hydrolyzates rich in branched amino-acids, glutamine, destined to clinical nutrition; hydrolyzates rich in sulphurated aminoacid destined to the nutrition of calves and suckling pigs, as well as hypoallergenic hydrolyzates destined to child nutrition.

#### BRIEF DESCRIPTION OF THE FIGURE

[0015] The only figure attached to this description represents an illustrative scheme for a specific execution of the invention process. Said scheme includes each stage and the material flow which should be followed to achieve a correct preparation process.

[0016] The numerical references have the following meaning:

1. Sunflower cake.
2. Protein extraction tank
3. Alkaline solution
4. Centrifuge
5. Soluble fraction
6. Soluble protein

7. Precipitation tank
8. Acid solution
9. Centrifuge
10. Atomizer
11. Dry isolate
12. Protein
13. Enzymatic reaction tank
14. Enzymatic solution
15. Hydrolyzate
16. Clarifier
17. Micro/ultrafiltration
18. Atomizer
19. Commercial hydrolyzate.

## EMBODIMENTS OF THE INVENTION

[0017] This invention is additionally illustrated by the following example, which does not intend to limit its scope.

[0018] In said example, the process used to obtain an enzymatic hydrolyzate of sunflower vegetable protein, with degrees of hydrolysis  $\geq 50\%$  is described in detail.

## EXAMPLE

[0019] The fractionation of degreased sunflower flour (HG) is the first stage of the process. By means of a flotation/sedimentation tower system, separation of the lignocellulosic fraction (FLC), soluble fraction (FS) and proteic fraction (FP) is performed. The proteic fraction obtained in the previous stage, is subjected, within a treatment tank, to an elimination process of the phenols present by rinsing with water: ethanol (90:10). Once this stage is completed, solubilization of the protein is carried out. To perform the latter, the proteic fraction, free from polyphenols is extracted in an aerobic medium with an alkaline solution. This stage uses an extraction tank with upper stirring, circuit breaker, reagent addition system, pH and temperature control. Likewise, a clarifying centrifuge and decanters are used.

[0020] The proteic extract obtained in an aerobic alkaline medium, is centrifuged and precipitated at its isoelectric point in a treatment tank with upper stirring, acid addition system and pH control. With the process described until now, the preparation is obtained, of the proteic isolate to be used as a substrate for enzymatic hydrolysis.

[0021] The isolate, at suitable concentrations, is hydrolyzed with alkalase until 12% hydrolysis and then kerase and flavourzyme are added. The proteolytic enzymes used are endopeptidases and exopeptidases. The degree of hydrolysis (GH) is determined by the pH-stat method described by Adler-Nissen (1986, Elsevier Appl. Sci. Publ.). The reactor used is of stainless steel, with systems for stirring, addition of water and reagents, as well as pH and temperature control.

[0022] Once finished the hydrolysis process, it is passed through a clarifying filter to eliminate solids in suspension and microemulsion. Then, it is fractionated by ultra-filtration to obtain customized peptides and finally, the material obtained is dried for its conservation.

MATERIALS FOR THE TOTAL PROCESS				
	Initial Weight	% Protein	Finished Kg	Y (%)
HG	5,000 Kg	25	1250	100
FP	1,500 Kg	50	750	60
Isolate	650 Kg	90	585	47
Hydrolyzate	468 Kg	95	445	36

## Claims

1. Process to obtain vegetable peptones with a high degree of hydrolysis, characterized because it includes the following stages:

a) To submit the vegetable protein isolate to a polyphenol elimination process.

b)

(i) To submit the polyphenol free proteic isolate to a first hydrolysis treatment with one or more endoproteases with non-specific activities, until obtaining an almost constant degree of hydrolysis of about 12-15%.

(ii) To submit the hydrolyzed product obtained in the previous stage to a second hydrolysis treatment with a mixture of more specific endoproteases and exoproteases until reaching degrees of hydrolysis equal or greater than 50%.

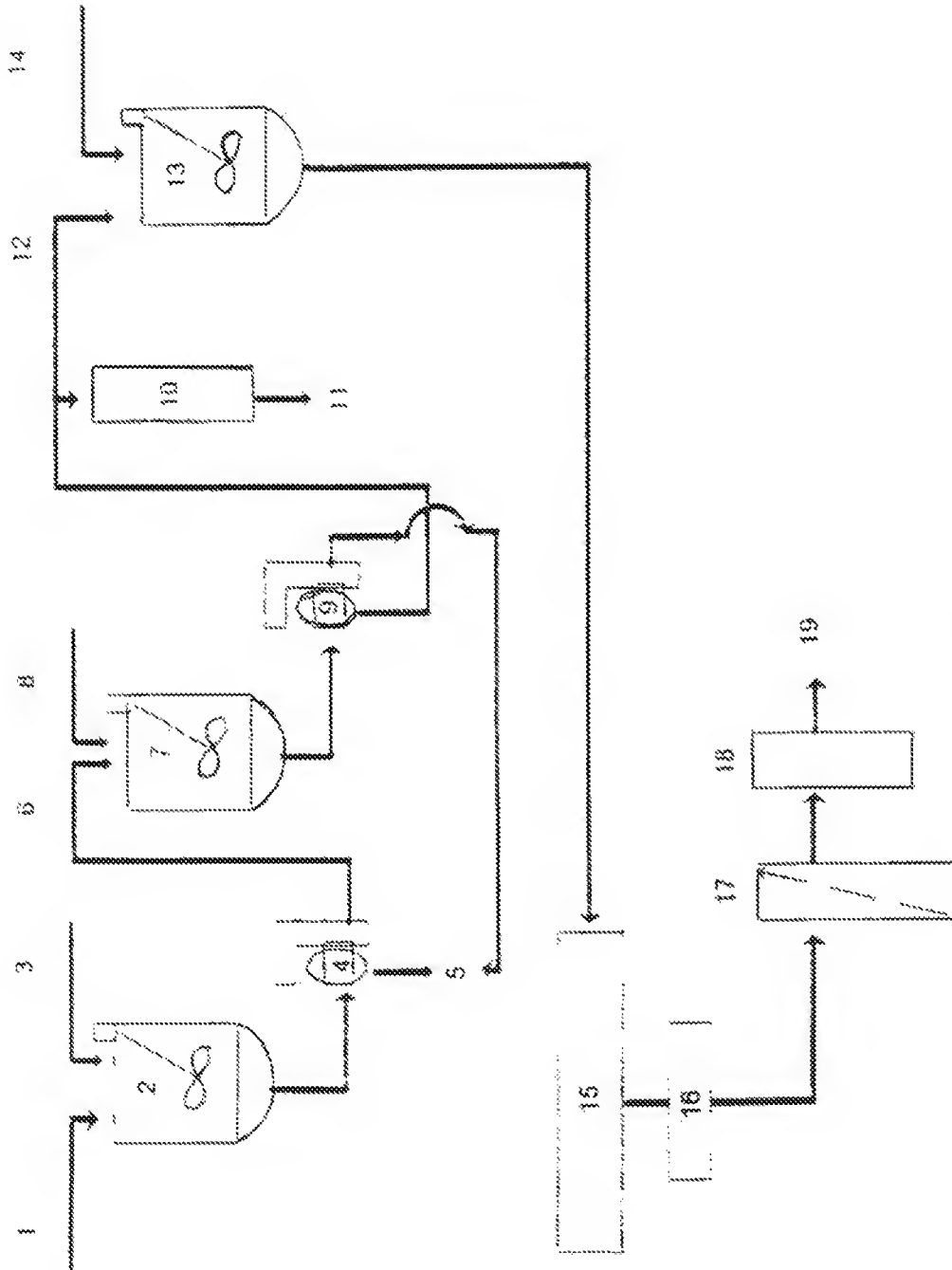
c) To concentrate and atomize the hydrolyzates obtained.

2. Process according to claim 1, characterized because the vegetable substrates are selected from among sunflower, rape seed, chickpea, lentil, lupin and grape refuse.

3. Process according to claim 1, characterized because the proteolytic enzymes are selected from among alkaliase, kerase, flavourzyme and L-600.

4. Vegetable peptones or vegetable hydrolyzates with a high degree of hydrolysis, obtained by the process of claims 1 to 3.

5. Use of peptones or vegetable hydrolyzates of claim 4, in the manufacture of nutritional products destined to clinical, animal and child nutrition.



# Index

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/ES 97/00294

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6: A23J3/34, 3/14, C07K1/12, A23L1/305, A61K38/01, A23K1/16

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6: A23J, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CIBEPAT, EPODOC, WPIL, CA, FSTA, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96/13174 A (NOVO NORDISK A/S) 09.09.96 see the whole document	1-5
Y	FARRADO, J. Et al., "Production of soluble enzymatic protein hydrolysate from industrially defatted nondehulled sunflower meal", 1991, J. Agric. Food Chem., 39, pp. 447-450, see the whole document	1-5
Y	WO 92/15697 A (NOVO NORDISK A/S) 17.09.92 see the whole document	1-5
Y	Base de datos WPIL en Questel, semana 9021, Londres, Derwent Publications Ltd., AN 90-162532 & SU 1500240 A (AS UZB PLANT CHEM) 15.08.89 abstract	1-5
A	WO 92/11771 A (NOVO NORDISK A/S) 23.07.92 see the whole document	1-5

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

\* Special categories of cited documents:

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"X" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"R" document member of the same patent family

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Authorized officer

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## INTERNATIONAL SEARCH REPORT

International application No

PCT/ES 97/00294

C (Continuation): DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	BAUTISTA, J. Et al., "Low molecular weight sunflower protein hydrolysate with low concentration in aromatic amino acids", 1996, J. Agric. Food Chem., 44, pp. 967-971 see the whole document	1-5
A	FARRADO, J. Et al., "Characterization of enzymatic sunflower protein hydrolysates", 1993, J. Agric. Food Chem., 41, pp. 1821-1825. see the whole document	1-5

Form PCT/ISA/210 (continuation of second sheet) (July 1992)